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Chelating Agents in Biological Systems

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Chelation enables metals to be transported to or from vulnerable target sites, and to hinder or facilitate their carcinogenic potential. In the reverse sense, metals are capable of ligand scavenging via complexation or mixed complex formation—the latter being the result of interaction with binary complexes. Consequently, metal complexes can be utilized for the transport of selected organic chemotherapeutic drugs to target organs, or for the decorporation of those toxic organic compounds which are able, before or after metabolic activation, of reacting with metals or 1:1 metal complexes. It is emphasized that the degree to which metal ions interact *in vivo* should employ the conditional constants which take into account competition from other ions, especially Ca^{2+}, H^+, and OH^-. The genotoxic consequences of the various chemical factors involved in chelation, along with examples: kinetics, stabilization of oxidation states, lipophilicity, and mixed ligand formation, are discussed.

Introduction

The transition metal ions inevitably exist as metal complexes in biological systems by interaction with the numerous molecules possessing groupings capable of complexation or chelation. Hence we find essential metals such as Cu, Zn, Cr, Fe, Mn, and Co existing as binary and ternary chelates of amino acids, carboxylic acids, and proteins. Without such interactions, life could not exist or be maintained, or, as earlier expressed, "Life could not exist or even come into being without the mediating action of metals" (*1*). Wood (*2*) made the cogent point when he wrote: ". . . metals are involved in every aspect of biosynthesis, biodegradation, and in the assembly of macromolecular structure. If you think that biochemistry is the organic chemistry of living systems, then you are misled: *biochemistry is the coordination chemistry of living systems.*" Certainly not all biochemical reactions involve metal compounds. On the other hand metals are involved in such a large number of essential biological reactions that life as we know it could not exist without coordination compounds.

The carcinogenic metals all have the ability—via complexation or ternary complex formation—to interact with DNA and other nuclear constituents or to alter such cellular properties as membrane integrity. Chelation phenomena enable metals to be transported to or away from vulnerable target sites, and to facilitate or hinder those intracellular interactions which may ultimately lead to cancer. In order to appreciate and apply the dual nature of metal chelation in carcinogenesis, both in its induction and therapy, I shall review selected aspects of the chemistry of metal ions, chelate formation, and the stability of metal chelates. A more comprehensive review of the chemistry of carcinogenic metals is given elsewhere by Martell (*3*). These chemical properties and resulting speciation all have a bearing on the ability of a metal ion to interact with target molecules. For example, while Cr(VI) as chromate, CrO_4^{2-}, enters cells, the ultimate mutagen or carcinogen from the chemical standpoint can only be Cr(III) which is the state which chelates with biological molecules as reference to the Sillén-Martell tables reveals (*4*). In fact, Cr(III) is not carcinogenic because it cannot penetrate cell membranes, but Cr(VI) is reduced to Cr(III) within the cell. On the other hand, chelation with exogenous chelating agents can reduce or prevent its mutagenicity (*5*). Similarly, results on the antitumor activity of acid ruthenium amine complexes suggest that reduction to Ru(II) occurs *in vivo* prior to metal coordination to nucleic acids (*6*).

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I will emphasize the ability of chelants to combine with and to transport metal ions—a process of metal scavenging. This property, especially when water-soluble, nonmetabolizable, and excretable chelates are formed, is widely applied for the decorporation of toxic metals, both stable and radioactive. A potentially useful "reverse" property is ligand scavenging in which the goal is to accelerate the excretion of toxic organic substances containing groupings capable of binding to a nontoxic metal prior to or after metabolic activation. Binary complexes may also combine with an additional ligand forming a mixed or ternary complex (*7*). This latter property appears to be the mechanism by which catecholamines (*8, 9*) are transported as well as nucleotides (*10*) and oxygen. In this connection, therefore, it would appear that metal complexes could transport organic chemotherapeutic drugs to target organs or remove excessive levels of the drugs.

Chelate Formation and *In Vivo* Stability

In biological systems important factors in the actions of metal chelates include: (1) intrinsic stability; (2) chelant-metal ratios; (3) stability as a function of pH; (4) competition from other metals and ligands, both endogenous and exogenous; (5) overall charge and lipophilicity; and (6) rates of hydrolysis and chelate formation. An excellent review of the chemistry of chelation and factors governing stability is provided by Mellor (*11*).

Chelating molecules possess at least two functional groups containing donor atoms capable of combining with the metal ion and so situated that a ring is formed with the metal ion. Since most chelants contain protons, these compete with the metal ion for the chelant:

$$M + H_nL \rightleftharpoons ML + nH^+ \qquad (1)$$

where L represents the ligand and M the metal ion. Generally we will focus on the binary 1:1 chelates and occasionally on 1:2 chelates, i.e., ML_2. The fraction of the metal present as a chelate, ML, derives from mass action:

$$ML/M = K_f(L) \qquad (2)$$

The equation shows that the fraction of M in chelate form, (ML)/[(M) + (ML)], is enhanced either by increasing the concentration of the ligand, or by a ligand producing a higher formation constant, K_f. Ligand concentrations, whether endogenous or exogenous, do not or cannot be varied greatly, e.g., most chelants such as EDTA or diethylenetriaminepentaacetic acid (DPTA) used for therapy of metal poisoning, become toxic above peak plasma levels of 10^{-3}–$10^{-4}M$. However, the values of K_f may range from 10^0 to $> 10^{30}$. Obviously, in the choice of chelants for biological use, the K_f values offer greater flexibility for the scavenging and release of bound metals or ligands.

When exogenous chelating agents are employed in biological systems, the stability of the binary complex as expressed by simple mass action does not necessarily reflect actual *in vivo* behavior. For example, in *in vivo* systems, Hg(II) is not bound by EDTA, despite the fact that the K_f of HgEDTA is very high, $\sim 10^{28}$. However, when competition from some of the common ligands in the plasma is considered, namely OH^- and serum albumin for Hg and H^+ and Ca^{2+} for EDTA, we find that there is practically no net binding by EDTA for the mercury. When calculations commonly employed in analytical and coordination chemistry are adapted to biological systems (*12-14*), they give the "conditional constant" which provides a more direct measure of metal chelate stability under biological conditions.

One example (*12*) of the usefulness of the conditional constant is the following: Fe(III)EDTA is about 10^6 times stronger then Cu(II)EDTA in conventional terms. Yet at pH 7, the Cu(II)EDTA chelate is more than 10^2 times stronger than Fe(III)EDTA (Fig. 1). This result, for example, has a bearing on the ease of dissociation *in vivo* of these chelates since they could result in the release of free toxic metal ions in vulnerable tissue or cells.

Rate Effects

The rates at which chelate or complex ion formation take place have important biological consequences, as, for example, in the manifestation or reversal of metal ion mutagenicity in microbial test systems (*5*). The rapid or labile reactions are usually completed by the time of mixing while the inert or robust systems may take minutes to days. While most metal chelates, at least of the divalent transition metals, Co, Cd, Mn, Zn, and Cu are labile, those involving Cr(III), Fe(III), Co(III), Mo(III), W(III), Mn(IV), Ru(II), Ni(II), and Pt(IV), are often inert (*11*). Outer orbital complexes(sp^3d^3) are usually labile and inner orbital (d^2sp^3) complexes are either labile or inert, the former when at least one *d* orbital is vacant, otherwise they are inert.

Hydrolyzable, polyvalent cations such as those of Cr, V, Ti, Zr, and Pu, undergo a complicated series of reactions with water and hydroxide ions, even-

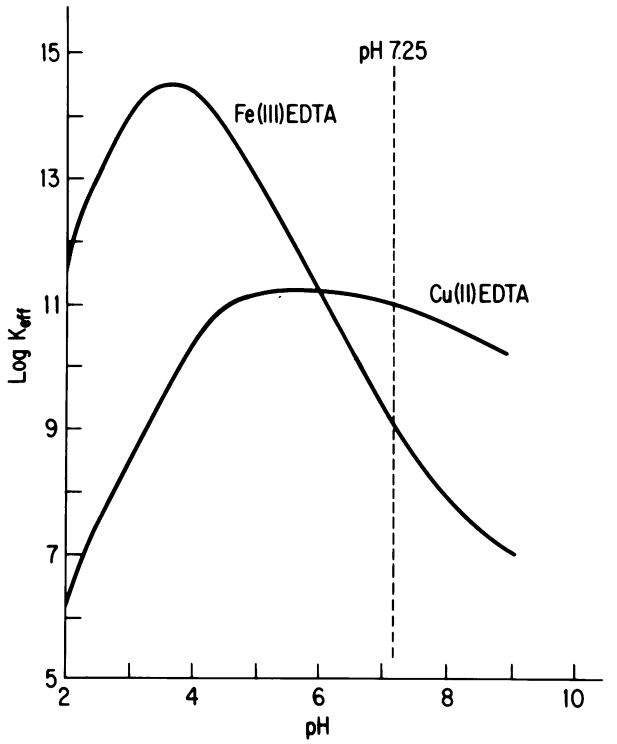

FIGURE 1. Variation of the log of the effective (conditional) constants with pH for the chelates of Fe(III)EDTA and Cu(II)EDTA. The calculations included competition from Ca^{++}, OH^-, and H^+. The curves are taken from a figure published elsewhere (*12*).

tually leading to inorganic polymers. Further, many ions, such as $Cr_2O_7^{2-}$ which cannot exist as such at pH > 5 will begin to change when simply dissolved in water. Thus, $Cr_2O_7^{2-}$ becomes chromate, CrO_4^{2-}, and $HCrO_4^-$. In fact, the genotoxicity of $Cr_2O_7^{2-}$ as measured in the rec-assay, decreased immediately after solution in water with half-life of 30 min (*5*). The biological consequences of slow conversions mean that different investigators may obtain apparently conflicting results, depending on the time elapsed between the preparation of the solution and the biological testing.

Mixed (Ternary) Complexes

In biological systems metal ions usually combine with two different ligands. For example, the binding of copper in serum by the normally occurring amino acids is greater than expected from their individual binding properties. It turns out that mixed complexes are formed in which a copper ion is bound simultaneously to two different amino acids. The biological significance of mixed complexes is becoming more and more recognized as described in the excellent review by Martin and Scharff (*15*). Perrin (*16*) employed a simulated plasma solution containing 22 amino acids and, by means of a computer program, showed that at pH 7.4 most of the copper was present as a mixed complex of Cu-histidine-cysteine, and that an appreciable fraction of zinc was found as a mixed complex of Zn-cysteine-histidine (Fig. 2).

In the presence of two chelants, the metal ion may form two independent binary complexes:

$$L + A + 2M \rightleftharpoons ML + MA \qquad (3)$$

Frequently the number of donor atoms or groups in a multidentate ligand is less than the coordination number of the metal ion. This permits another ligand to occupy these sites which would otherwise contain solvent molecules. Formation of the mixed complex usually occurs stepwise as follows:

$$L + A + M \rightleftharpoons L + A \rightleftharpoons MLA \qquad (4)$$

where ligand L has a higher denticity than A, which is usually bidentate. However, both ML and MA are present to some extent, the relative fractions of each would depend on kinetics and relative stabilities. Large, highly charged anions such as $EDTA^{4-}$, cannot accommodate two such molecules to give ML_2. However, even though the hexadentate EDTA may occupy all of the coordination sites, another, lower dentate ligand can displace one or more of the coordinating carboxyl groups. With Cr(III), the EDTA chelate is quinquedentate with a coordinated H_2O in the sixth position (*18*). The coordinated H_2O is labilized by the free carboxylate of EDTA and easily replaced by acetate or azide to form the mixed complex $Cr(EDTA)X^{(n+1)}$. The displacement of ligand groups during mixed complex formation can lead to important new properties not normally possible in the parent binary complex, e.g., formation of protonated species and ligand-ligand interactions within the coordination sphere (*19*).

When a second ligand, A, occupies uncoordinated sites in a binary complex, an important correlation ensues, namely that the stability of the mixed ligand chelate is greater, the larger the difference between the log K_1/K_2 values [the K_f in Eq. (2)] of the binary complexes (*20*).

Statistically, a metal ion prefers or favors combination with different ligands rather than two of the same (*21*, *22*). However, the stability of some mixed complexes exceed that expected from the statistical factor by many orders of magnitude.

It is interesting to note that in biological systems the imidazole moiety of histidine enhances the stability of mixed complexes when an -O- ligand

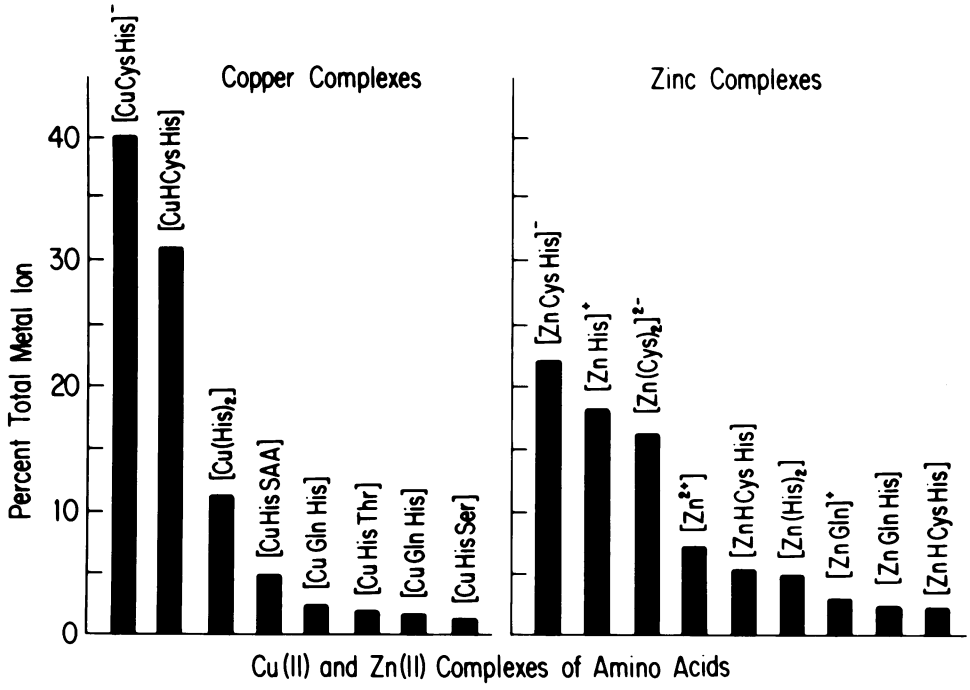

FIGURE 2. Calculated distribution of Cu(II) and Zn(II) among 22 amino acids; $Cu^{2+} = 2.7 \times 10^{-7} M$ and $Zn^{2+} = 3.3 \times 10^{-6} M$. The amino acid concentrations were those known to exist in blood plasma. SAA is a composite representing alanine + arginine + isoleucine + leucine + valine. The bar graphs were constructed from data tabulated by Perrin and Agarwal (*17*). Altogether 19 copper species were estimated to exist, accounting for 98% of the total copper, while 20 zinc species accounted for 93% of the total zinc. In the case of copper, about 86% of all the species were mixed ligand complexes, while about 40% of the zinc species were mixed ligand complexes.

atom is involved (*19*). With synthetic chelants such as nitrilotriacetic acid (NTA) and imidazole, transition metals form strong mixed complexes. In naturally occurring mixed complexes with proteins, the imidazole moeity from histidine together with -O- donor atoms is ubiquitous (e.g., hemoproteins and the non-heme hemocyanins).

Oxidation-Reduction

Chelation serves to stabilize that oxidation state of a metal ion forming the most stable chelate with the particular chelant. For example, an unusual oxidation state of Ag^{2+} can be stabilized by chelation with 2,2′-dipyridyl and that of Ni^{3+} by chelation with o-phenylenedimethylarsine (*11*). The stability of a metal complex to oxidation-reduction is a function of the stability of the metal complex. These are important considerations in biological systems when it is desired to use or maintain a given oxidation state.

Biological Consequences and Applications

I have already alluded to several biological roles of chelation. In this section I will cite several additional ones useful for biomedical purposes.

Lipophilicity and Intracellular Transport

In the classical work of Albert on the antimicrobial activity of the Fe(II) and Cu(II) chelates of 8-hydroxyquinoline and their derivatives (*23*), only the neutral 1:2 chelate was able to pass through the cell membrane. Within the cell the 1:2 chelate dissociated into the 1:1 chelate which was the actual antibacterial agent, presumably by forming a ternary complex with essential enzymes. Similarly, with Fe(III), only the neutral 1:3 chelate could penetrate the cell, where again dissociation took place. It is possible, as Gershon and co-workers have shown (*24*), to replace one of the 8-hydroxyquinolines in the 1:2 chelate of copper with bidentate arylhydroxy acids such as salicylate. The resulting mixed ligand complex is also neutral and antibacterial. The cobalt(II)-EDTA chelate can combine with cyanide, and, in fact, the mixed complex has been recommended and used as an antidote cyanide poisoning (*16*).

As a means of enhancing intracellular penetra-

tion by synthetic chelating agents, esterification of the functional groupings, such as the carboxyls in EDTA, have been accomplished on the assumption that the active ligand groupings within the cell are released by hydrolysis of the ester. In fact, such compounds have been effective in removing heavy metals such as Ce, Y, and Pu from the liver. Unfortunately, these esterified compounds have a low therapeutic index (*14*).

It is feasible to use metabolic inhibitors to jam metabolic reactions such as the tricarboxylic acid cycle. For example, Pb reacts with citrate to form a moderately strong chelate but parenteral administration of citrate has little effect because of the rapid metabolism of the citrate. When sublethal doses of sodium fluoroacetate are given to rats, the citrate levels increase 20-fold or more within the spleen, kidney, and liver. In fact, fluoroacetate administration caused a small but significant (15%) decrease in the acute toxicity of Pb (*25*).

Catalase-like Activity

The decomposition of H_2O_2 is catalyzed by chelated metal ions though the rate is not as efficient as the iron-containing enzyme, catalase, in terms of the number of molecules of H_2O_2 decomposed per unit time. The mechanism of catalysis by synthetic chelates, which is far greater than that due to the uncomplexed metal ion, utilize the chelating properties of peroxide which acts as a bidentate ligand, HOO^-, as described by Wang (*26*). Copper chelates such as Cu - histamine and Cu - histidine are good catalysts at pH 7. The mechanism involves again the chelating property of HOO^-. It was shown (*27*) that two adjacent coordination sites of the Cu(II) must be available. Hence, if the quadridentate copper is chelated by a tridentate or tetradentate chelant, no catalytic property is observed.

Viral Interactions

Metal chelates which contain vacant coordination sites appear to combine with a sulfur bridge of the protein coating of a virus. This, as proposed by Eichhorn (*28*) changes cleavage, enabling the DNA to leave the virus (*15*). Virus T_2 bacteriophage infect *E. coli* by a similar mechanism. The DNA passes from the phage to the bacteria, an essential step in viral replication. $Cd(CN)_2$ appears to act by the formation of a bond between the metal complex and a sulfur bridge of the protein coating of the virus. With virus T_2 bacteriophage it is believed that a zinc complex in the cell wall of the bacterium exists and is bound to a sulfur-containing bond of the protein coating which is then destroyed. Note that these proposed mechanisms involve ternary complex formation.

Miscellaneous

Complexes and chelates of platinum(II) are proving to be effective anticancer agents. The *cis*-dichlorodiammineplatinum, a mixed complex, is finding widespread clinical applications as described by Rosenberg (*29*) in a significant review. Other active complexes of Pt include those in which one of the ligands is bidentate, such as dichloroethylenediammineplatinum. It is conceivable that these Pt compounds interact with DNA via ternary complex formation.

Mixed complex formation may be utilized also as a ligand scavenger to transport ligands or hasten their elimination, as mentioned earlier. This phenomenon could, in principle, utilize nontoxic binary chelates for the transport of chemotherapeutic drugs to target sites, or to detoxify poisonous substances including pesticides which possess functional groupings capable of chelation or, which acquire such groupings during metabolism. Of interest is the role of metal and ligand scavenging in the induction or reversal of mutagenic effects (*5*).

Metals and their complexed forms play important roles in various diseases (*30*). For example, copper and copper complexes have been postulated to play a role in inflammatory processes (*31*). In a series of papers, Williams and coworkers (*32*) have reviewed and analyzed the role of metal complexes in rheumatoid arthritis, especially those of ternary copper complexes.

It is tempting to conclude that the so-called role of metals in carcinogenesis reflects the transport of the binary and ternary complexes of the metals to and from intracellular target sites.

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